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New microextraction methods for the evaluation of bromohexine HCl in pure and pharmacological formulations.

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Abstract

In this study, ion-pair reactions were used to investigate bromohexine HCl. The development of simple, accurate, sensitive, low-cost, and efficient extraction methods for bromohexine HCl separation, such as the DLLME and cloud point extraction techniques, are described as bromohexine HCl estimation methodologies. These methods employed the interaction of bromohexine HCl with alizarin yellow reagent to produce a yellow complex in an acidic medium (pH = 5). The complex's maximum absorbance intensity was 480 nm, and the stoichiometry for both continuous variation and molar ratio methods was determined to be 1:1. The dispersive liquid liquid microextraction (DLLME) method's concentration range (1-23µg.mL^{-1}), the Beers law was obeyed with correlation coefficient (R^2=0.998), limit of detection as (0.055µg.mL^{-1}), limit of quantification as (0.183µg.mL^{-1}) and molar absorptivity as (23930.2L.mol^{-1}.cm^{-1}).In the second technique, the cloud pointextraction method, The linearity of calibration curve above was the range between (1-40 µg.mL^{-1}), the correlation coefficient (R^2=0.998) and molar absorptivity was (13202.88L.mol^{-1}.cm^{-1}), (LOD) and (LOQ) were (0.141µg.mL^{-1}) and (1.4641µg.mL^{-1}), respectively. The proposed techniques can be used for the determination of bromohexine HCl in both pure and pharmaceutical formulations with very good success.

Key word: Bromohexine HCl (BRH), DLLME, Cloud point extraction, spectrophotometer.

1. Introduction

BromohexineHCl (BRH), known as 2, 4-dibromo-6-[[cyclohexyl (methyl) amino] methyl] aniline; hydrochloride[1]. Molecular Formula **$C_{14}H_{20}Br_2N_2.HCl$** (Figure.1)[2]

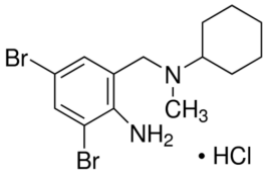

Figure.1*: Bromohexine HCl structure.*

BromohexineHCl is a white crystalline powder ,It is also somewhat soluble in chloroform and methylene chloride[1].Bromhexine is a benzyl amine-derived cardiac depressant of vasicine that is generated from the plant Adhatoda vasica[3]. It is an expectorant that reduces the viscosity of the material, making it easier to cough up and dispose [4].The mechanism of action is based on sputum decomposition and dark coughing; Respiratory production helps in the formation of thinner, less thick phlegm[2]. Assisting vasomotor secretion generates a vasomotor secretory effect[5]. Several analytical techniques were used to estimate bromohexine HCl in medicinal formulations such as Potentiometric Flow Injection[6], HPLC-ICP-MS [7], HPLC[8], Thin Layer Chromatography (TLC)[9], Spectrophotometric Quantitative[10], and UV-Vis Spectrophotometer[11][12][13]. The dispersive liquid liquid microextraction (DLLME) and cloud point extraction have many advantages in the determination of pharmaceutical preparation like rapid, safety and low cost[14].In this work, the proposed technique is based ion-pair reaction of bromohexine HCl with alizarin yellow reagent in the acidic medium, then evaluation and pre-concentration

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using (DLLME) and cloud point extraction. Using two different methods , the aim of this study is to describe, determine , and identify the optimum conditions for determination bromohexineHCl medicines using dispersive liquid liquid microextraction (DLLME) and cloud point extraction, then compare between the two methods.

2. Experimental

2.1. Materials

All spectra and absorption intensity measurements were done using a double beam UV-Vis spectrophotometer with 1cm quartz cells.A Metrohm 780 digital pH meter (Switzerland) with a combination glass electrode was used to take all of the pH measurements. Double distilled water was used throughout the experiments. During the extraction process, an IKA clever 3 vortex mixer (Staufen, Germany) was used. The organic and aqueous phases were separated using a Hermle Z-300 centrifuge (Wehingen, Germany). The chemicals bromohexine HCl from SDI Samarra, hydrochloric acid from Scharla , alizarinyellow from sigma-Aldrich, acetic acid , chloroform , ethanol , methanol ,carbon tetrachloride were purchased from BDH(England) .

2.2. Methods.

A 500µg/mL stock solution of bromohexine HCl was prepared by dissolving 0.05gm of BRH in10 mL of 0.1N HCl and making up to 100mL with double distilled water in a volumetric flask. Phosphate buffer[15][16] was prepared by the addition of (45.2mL) of 0.1M sodium hydroxide to (100mL) of potassium hydrogen phthalate 0.1M to adjust the pH at 5.0 and the mixture is brought up to 100mL with double distill water .

2.3. Pharmaceutical preparations procedure.

Three concentrations of bromohexineHCl in Solvodin5,10, and 15µg.mL^{-1} were taken, and treated in the same way as cloud point extraction in pure drugs, and three concentrations of bromohexine HCl in Solvodin 3,5, and 7 µg.mL^{-1}were extracted using the same method as DLLME, the absorbance was measured at λ_{max} 480nm.Bromohexine Hydrochloride in Biosolvon were treated in the same way as cloud point extraction and DLLME techniques in pure drugs, and the absorbance was measured at a wavelength of 480nm.

2.4. General procedure of DLLME for amino medications[17].

20 µg. mL^{-1} of each of the drug and the reagent were prepared, and 0.5 ml of drug and1 mL of alizarin yellow reagent were put to a 15 mL glass centrifuge tube , and 0.8 mL of acetate solution (PH = 4) were added and complete to 10mLl distill water. A cloudy solution was created by rapidly injecting 400µL chloroform as an extraction solvent and 700µL ethanol as a dispersive solvent into the solution using a micro syringe. For 6 minutes, the mixture was centrifuged at 2000rpm. A micro syringe was used to obtain the yellow ion pair complex, and the absorbance at 480nm was measured against a blank.

2.5. General procedure of cloud point extraction (CPE) for amine medications[18].

A 0.5mL standard drug solution was transferred to a 10mL glass centrifuge tube stoppered tube and 1.5 mLof phosphate buffer (pH = 5) was added to it, then 2 ml of alizarin yellow reagent was added.Then added 0.8 mL of tritonX-114 and completed the volume with double distilled waterto reach 10 mL and placed in a water bath at 50Cº for 20 minutes. After using a centrifuge at 3000 for 4 minutes to separate the two phases. The cloud was separated and dissolved in 2mL of methanol. The absorbance of the colored solution was scanned on spectrophotometer in the range of 300-700nm against a drug-free blank solution.

3. Results and discussion

When the BromohexineHCl cation (BRH +) binds to the yellow Alizarin reagent anion (A-), a yellow colored ionic pair compound is generated (A^- _ BRH^+). The yellow complex's absorbance can be measured using a spectrophotometer in pH 5 at λ_{max} 480nm against a blank; the spectrum is shown in the figure2.

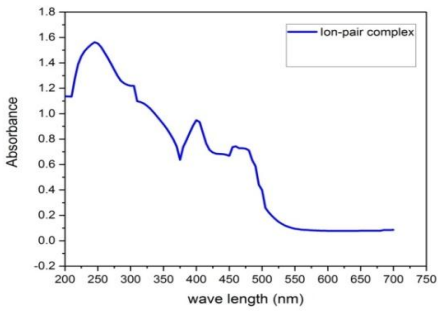


Figure2: Absorption Spectrum of the Resulting complex.

3.1 Optimization of DLLME

The ion-pair complex of bromohexine HCl was extracted using the DLLME technique, and its spectra were analyzed at 480 nm. In the DLLME combine with a UV-visible spectrophotometer was used to select the best conditions for the complexity of bromohexine HCl drug and an alizarin yellow reagent. The effect of the extraction solvent (chloroform, carbon tetra chloride, benzene and hexane) was investigated (Table.1).

The solvent that has been distributed (ethanol, methanol, acetone, and acetonitrile) was studied (Table.2). The optimum extraction and dispersion solvents for complex formation were chloroform and ethanol, according to the results.

Table.1: Selection type of extraction solvent

Type of extraction solvent	Absorbance (λ_{max}480nm)
Chloroform	0.641
carbon tetra chloride	0.611
Benzene	0.527
Hexane	———

Table.2: Selection type of dispersive solvent

Type of dispersive solvent	Absorbance (λ_{max} 480nm)
Methanol	0.642
Ethanol	0.639
Acetone	0.620
Aceto nitrile	0.539

The pH was also investigated; the pH range of the phosphate buffer employed was between (1-8). It was also discovered that pH = 5 provided the optimum pH for the production of the complex. (Figure.3).

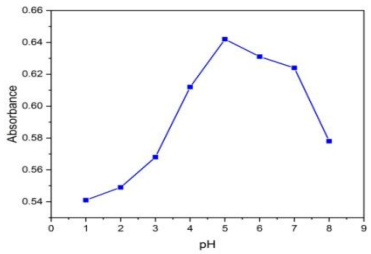


Figure3: *Effect of PH buffer*

A variety of buffer solutions (phosphate, acetate, and citrate) were tested and it was observed that the phosphate buffer produced the maximum absorption value (Table.3).

Table.3: Effect of buffer type

Buffer type	Absorbance (λ_{max} 480nm)
Acetate buffer	0.582
Phosphate buffer	0.641
Citrate buffer	0.561

The absorption values of various volumes of phosphate were investigated, and it was discovered that the volume of 1.2 mL recorded the greatest absorption value at 480nm. (Figure.4).

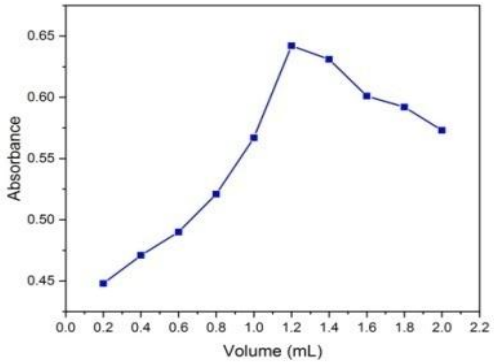


Figure4**:** *Phosphate buffer volume*

The complex formation between bromohexine HCl and alizarin yellow reagent is best in a volume of 1.5 mL of the reagent and is sufficient for complex formation (Figure.5).

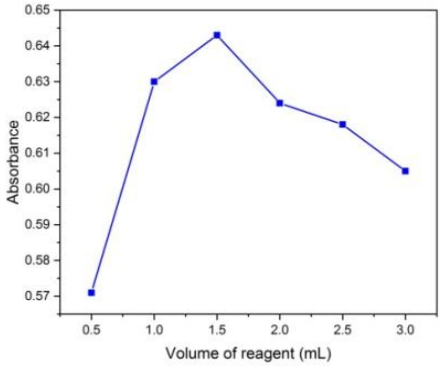


Figure5: *Effect concentration reagent*

The best volumes for both the extraction and dispersion solvents were found to be 300 mL and 900 mL, respectively (Table.4&5).

Table. 4: *Effect of the extraction solvent volume*

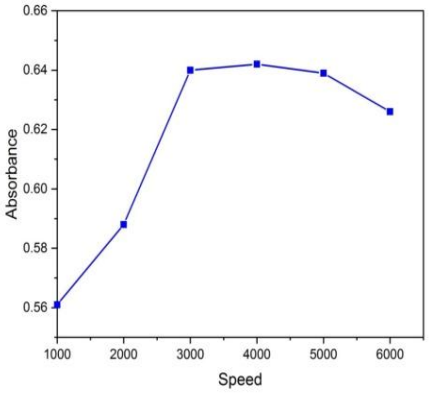
Extraction solvent volume(mL) (chloroform)	Dispersive solvent volume(mL) (Ethanol)	Absorbance (λ_{max} 480nm)
200	700	0.529
300		0.640
400		0.639
500		0.570

Table 5: Effect of the dispersive solvent volume

Extraction solvent volume(mL) (chloroform)	Dispersive solvent volume(mL) (Ethanol)	Absorbance (λ_{max} 480nm)
300	500	0.520
	600	0.563
	700	0.590
	800	0.601
	900	0.643
	1000	0.638
	1100	0.571
	1200	0.562
	1300	0.533
	1400	0.521
	1500	0.520

The effect of speed and time in the centrifuge plays an important role in the extract and separate of complex. The best speed and extraction time were 6 minutes and 4000 rpm (Figure. 6&7).

Figure 6: *Effect of the centrifuge speed*



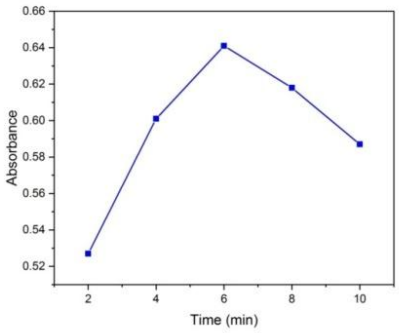


Figure 7. *Effect of the centrifuge time*

The effect of time on extraction was studied using a various times ranging from 1 to 20 minutes, with color stability reported even after 20 minutes (Table.6).

Table .6: *Effect of the extraction time*

Time(min)	Absorbance (λ_{max} 480nm)
1	0.641
2	0.643
4	0.640
6	0.643
8	0.644
10	0.640
12	0.643
14	0.642
16	0.643
18	0.643
20	0.642

Carbohydrates such as glucose, fructose, lactose, and other sugars that are added to pharmaceutical formulations have no effect on the drug. (Table.7).

Table .7: *Extraction recovery with different interference compound*

Interference	Recovery%
Starch	98.1
Glucose	96.9
Maltose	98.4
Lactose	97.5
Glysin	98
Fructose	98.3

3.2. DLLME Calibration Curve

The calibration curve was created by plotting absorbance against bromohexine HCl concentration. The concentration ranged from 1 – 23 µg.mL^{-1}. The linear calibration equation for bromohexine HCl is Y=0.058X-0.015 and R^2=0.998 of the linear calibration (Figure.8)

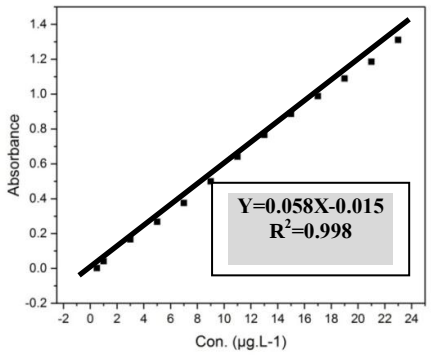


Figure8: *Calibration curve for DLLME*

3.3. Optimization of cloud point.

The pH was investigated, with the pH of the phosphate buffer employed ranging from 1 to 8. It was also discovered that pH = 5 provided the optimum pH for the production of the complex (Figure9).

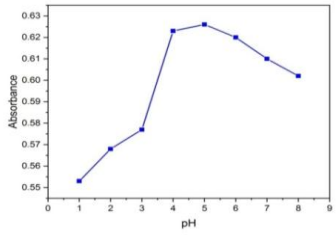


Figure9: *Effect of pH*

Type of buffer	Abs.λ_{max} 480 nm.
Acetate	0.618
Citrate	0.504
Phosphate	0.625

Table 8: *Type of buffer*

phosphate, acetate, and citrate buffer solutions were tested, and it was observed that the Phosphate buffer generated the highest absorption value. (Table.8). When several amounts of phosphate were tested, it was discovered that 0.8 mL had the greatest absorption value at 480nm (Figure10).

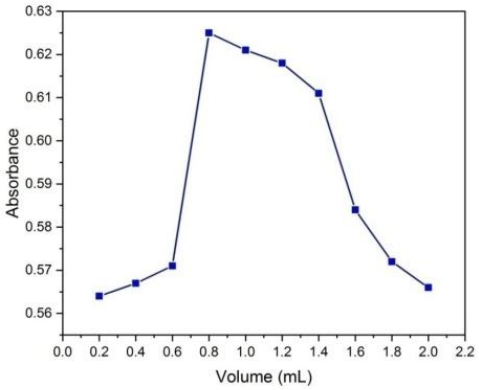


Figure10: Effect *of volume phosphate buffer*

A variety of surfactant solutions such as Triton X-114, Triton X-100, Tween20, CATB and SDS were investigated, and it was found out that the Triton X-114 produced the maximum absorption value (Table.9).

Table.9: *Effect of type of surfactant*

Type of surfactant	Absorbance λ_{max} 480 nm
Triton X-114	0.626
Triton X-100	————
Tween 20	0.412
CATB	————
SDS	————

The different volumes of surfactant were tested, and it was observed that a volume of 0.8 mL was recorded the highest absorption value at 480nm (Figure.11).

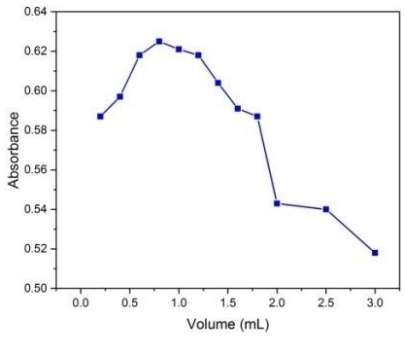


Figure11: *Effect of surfactant volume*

Temperatures ranging from 30 to 80 Cº were studied using a water bath, and it was showed that 50 Cº had the highest absorption value at 480 nm. (Figure12).

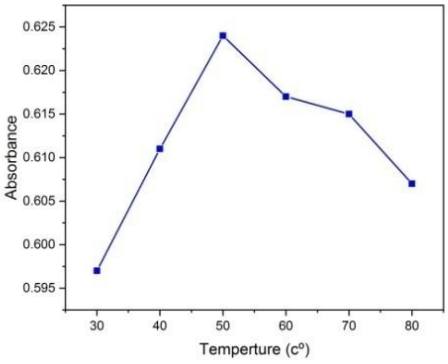


Figure12: *Effect of Temperature in water bath*

The time necessary for extract and separate the complex was measured using a water bath and ranged from 10 to 60 minutes, with 40 minutes containing the greatest absorption value at 480 nm (Table.10).
The effect of time in the centrifuge plays an important role in the isolation and extraction of complex. The best time extraction was 5 minutes (Table.11).

Time (min)	Absorbance λ_{max} 480 nm
10	———
20	———
30	0.618
40	0.625
50	0.621
60	0.619

Table.*10: Effect of incubation time (min)*

Time (min)	Absorbance λ_{max} 480 nm
1	———
2	———
3	———
4	0.622
5	0.624
6	0.620

Table*.11: Effect of centrifuge time (min)*

The effect of speed on the extraction of complex in the centrifuge is crucial. 5000 rmp was the highest extraction speed.(Table12.).

Centrifuge rate(rmp)	Absorbance
1000	———
2000	———
3000	0.611
4000	0.620
5000	0.624
6000	0.617

Tab*le 12: Effect of centrifuge rate (rmp)*

The effect of several solvents (Methanol, Ethanol, Chloroform, and Hexane) on absorbance of complex was studied; Ethanol was shown to be the best solvent for obtaining the highest absorbance (Table.13).

Table.*13: Select of best solvent*

Solvent	Absorbance λ_{max} 480 nm
Ethanol	0.624
Methanol	0.620
Chloroform	0.518
carbon tetra chloride	0.501
Hexane	———

Table 14 shows that interference that may be added to pharmaceutical preparations, such as (glucose, fructose, lactose, etc.) had no effect on the medicine

3.4. Cloud point Calibration Curve.

Table14: *Extraction recovery% with different extraction*

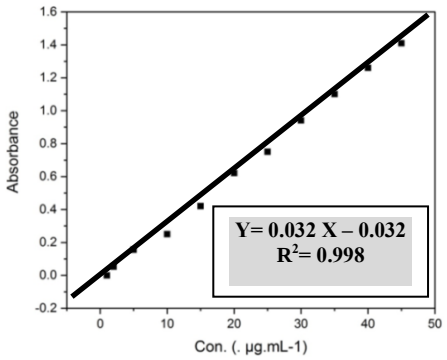
Interference	Recovery%
Starch	99.04
Glucose	99.36
Maltose	100.3
Lactose	98.1
Glycine	96.8
Fructose	99.7

The calibration curve was constructed using the absorbance against concentration of bromohexine HCl. The concentration range was between (1-40µg.mL^{-1}).The regression equation of bromohexine HCl is Y=0.032X-0.032 and R^2=0.998 of the linear calibration (figure13).

3.5. Accuracy and precision for DLLMEand cloud point method

All measurements in the proposed approach were evaluated using the bromohexine HCl calibration curve. In order to achieve DLLME and cloud point accuracy, the average drug was determined, indicating the accuracy of the technique. Using three different concentrations, the relative standard deviation was calculated, and the recoveries indicated accuracy and reproducibility. The results were appropriate for the proposed method**.**

Figure13: *Calibration curve for Cloud point interference compoun*



Conclusion

DLLME and cloud point extraction both use bromohexine hydrochloride (BRH) and alizarin yellow reagent to extract a bright yellow ionic molecule, as well as UV-Vis Spectrophotometry. Bromohexine hydrochloride has been extracted and quantified using the recommended DLLME and cloud pointextractionapproaches in both pure and pharmaceutical formulations**.**

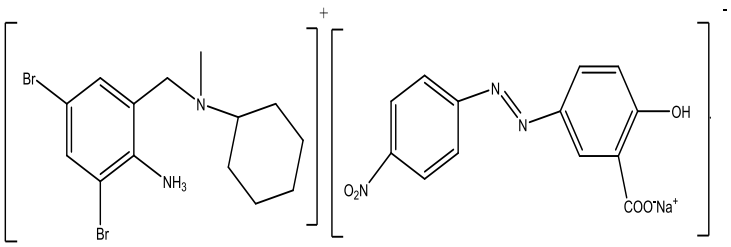


Figure14 *. The structure of ion complex of BRH*

Table.15: *Analytical and statistical parameters of DLLME and cloud point extraction methods.*

*CPE (X1=5, X2=10, X3=15) * DLLME (X1=3, X2=5, X3=7)

Parameters	DLLME	CPE
λ_{max} nm	**480**	
Color	**yellow**	
Regression equation	**Y=0.058X-0.015**	**Y=0.032X-0.032**
Linearty range(µg/mL^{-1})	**1 – 23 µg.mL^{-1}**	**1-40µg.mL^{-1}**
Correlation Cofficient (R^2)	**0.998**	**0.998**
Ɛ(L.mol^{-1}.cm^{-1})	**23930.2**	**13202.88**
Sandell'ssensivity (µg . cm^{-2})	**0.0172**	**3.121×10-3**
Slope (b)	**0.058**	**0.032**
Intercept(a)	**0.015**	**0.032**
Limit of detection(µg/mL^{-1})	**0.055**	**0.141**
Limit of quantification(µg/mL^{-1})	**0.183**	**0.4641**
C.L.for the slope(b±ts$_b$) at 95%	**0.058 ± 0.2249**	**0.032 ± 6.5×10-3**
C.L.for the intercept(a±ts$_a$) at 95%	**1.1825 ± 0.015**	**0.032 ± 0.487**
Standard error for regression line ($S_{y/x}$)	**0.1479**	**0.2263**
***C.L for Conc.X$_1$µg ml^{-1}at 95%**	**3.14 ± 2.48×10^{-3}**	**4.8 ± 2.5×10-3**
***C.L for Conc.X$_{2µg}$ ml^{-1}at 95%**	**4.85 ± 2.48×10^{-3}**	**9.7 ± 5×10-3**
***C.L for Conc.X$_3$µg ml^{-1}at 95%**	**6.7 ± 2.48×10-3**	**14.8 ±2.5×10-3**

Table.16: *Application of the proposed DLLME and cloud point extractionfor the evaluation of bromohexine HCl*

drug	**DLLME method**					
	Conc. of drug mg.L^{-1}		**Relative Error%**	**Recov. %**	**Average Recov%**	**RSD% (n=3)**
	Taken	Found				
Solvodin	3	2.76	8	92	96	0.02
	5	5.1	-2	102		0.02
	7	6.6	5.7	94		0.02
Biosolvon	3	2.8	6.6	93.3	96.2	0.02
	5	4.7	6	94		0.02

	7	7.1	-1.4	101.4		0.014
Cloud point extraction method						
Solvodin	5	4.8	4	96	97.2	0.02
	10	9.7	3	97		0.021
	15	14.8	1.3	98.6		0.7
Biosolvon	5	4.7	6	94	97.8	0.02
	10	10.2	-2	102		0.02
	15	14.6	2.6	97.3		0.7

Table.17: *Comparison the values of Linearity, LOD and Recovery of various methods reported in literature*

Method	**Linearity µg.mL^{-1}**	**LOD µg.mL^{-1}**	**Recov%**	**Ref.**
Potentiometric Flow Injection	$3.16x10^{-5}$-$1.00x10^{-2}$	------	98.2-99.8%	13
HPLC	0.391–100	0.195	97.88 - 100.68	[19]
HPLC	10-60.0	5	95.3	[20]
Thin Layer Chromatography(TLC)	4-40	0.521	98.67	[9]
Spectrophotometric Quantitative	2-20	0.2011	99.63	[10]
UV-Vis Spectrophotometry	2-14	-------	100.083	[5]
UV spectrophotometric	2.5-25	1.65	100.47	[2]
	2.5-25	2.12	99.84	
	2.0-25	2.58	99.57	
UV-Vis Spectrophotometry	1-12	0.481	-97.66 98.7	[4]
DLLME	1-23	0.055	96.1	Present work
Cloud point	1-40	0.141	97.5	Present work

4. Acknowledgments

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